Evaluation of the bacteriological and clinical profile of chronic pharyngitis with emphasis on studying the potential role of atypical pathogens as etiological agents

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CERTIFICATION

This is to certify that the dissertation entitled, **"Evaluation of the bacteriological and clinical profile of chronic pharyngitis with emphasis on studying the potential role of atypical pathogens as etiological agents"** is the bonafide work of **Dr. Naina Emmanuel** toward the M.S. Branch-IV (Otorhinolaryngology) Degree examination of the Tamil Nadu Dr. M.G.R. Medical University, to be conducted in March 2010.

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AIMS AND OBJECTIVES

1. Evaluate the clinical and bacteriological profile of chronic pharyngitis in adults.

2. To establish the role of atypical bacteria in chronic pharyngitis.

Abstract

Evaluation of the clinical and microbiological profile of patients with chronic pharyngitis with special emphasis on role of atypical organisms

Objectives/Hypothesis:

To study the clinical and microbiological profile of patients with chronic pharyngitis with special emphasis on role of atypical organisms such as *Chlamydia pneumoniae,Mycoplasma pneumoniae* and *Fusobacterium necrophorum*.

Study design:
A prospective case control study.

Methods:
344 patients with chronic pharyngitis were clinically evaluated to exclude non infectious causes of pharyngitis. Of these, 71 patients in whom comprehensive otorhinolaryngological examination did not elicit any factor contributing to the chronic pharyngeal complaint were recruited as cases and healthy asymptomatic adults were taken in the control arm. Both groups underwent throat swab for aerobic and anaerobic culture and ELISA to detect IgA antibodies against *Chlamydia pneumoniae* and *mycoplasma pneumoniae*.

Results:
Thirty-five of the 71 patients (49.3%) and 16 of the 71 control subjects (22.5%) were found to be seropositive for *Chlamydia pneumoniae*. Anaerobic organisms including *Fusobacterium spp.* were found to be significantly lower in cases as compared to controsl ($p = 0.041$).There was no significant difference between cases and controls in aerobic culture growth and seropositivity to *Mycoplasma pneumoniae*. Patients with chronic pharyngitis were found to have a significantly higher rate of *C.pneumoniae* seropositivity than the control group ($p = 0.001$).

Conclusions:
Infection with *Chlamydia pneumoniae* seems to be a significant etiological factor for chronic pharyngitis.

INTRODUCTION

Sore throat is a common clinical condition seen by general practitioners and ENT physicians. According to western figures approximately10% of the population present to the general practitioner with throat complaints for which a diagnosis of pharyngitis is made[1]. Pharyngitis accounts for over 40 million visits by adults to medical facilities each year in the United States where it accounts for over 100 million days of absence from the workplace annually[2]. The impact of this disease condition in the Indian scenario has not been ascertained.

In all otorhinolaryngology outpatient clinics the most common complaint among patients is persistent sore throat and the most frequent diagnosis is chronic pharyngitis with the underlying etiology unclear in most cases[3]. Despite numerous consultations and countless investigations they often fail to achieve relief of their symptoms .These patients are evaluated for a possible infectious etiology failing which an irritant cause is contemplated. Pharynx being a common conduit for the nasal, oral and respiratory passages is exposed to panoply of irritants which can incite pharyngeal inflammation. Various possible etiological factors suggested are heavy smoking, occupational irritants, sinusitis with post nasal drip, mouth breathing, acid reflux and poor dental hygiene .Elimination of the inciting cause and treatment of infection with antibiotics are suggested remedies. However despite detailed evaluation and many diagnostic procedures, the most common scenario is a patient with persistent sore throat and no discernible aetiology.

Recently there have been reports of agents such as *Chlamydophila pneumoniae*, *Mycoplasma pneumoniae,* and anaerobic bacteria such as *Fusobacterium necrophorum*, which

are potentially treatable by currently available antibacterials – playing a role in chronic pharyngitis .However it has not been established whether they simply act as co-pathogens or as primary etiological agents [4-6].

Though this diagnostic dilemma exists and is a significant source of morbidity and of course anxiety, there is a paucity of literature on this subject especially from India. Therefore, keeping this in mind we undertook a prospective study to look for other etiological agents in this condition. Elucidation of etiological agents can help in better understanding of the disease condition and better management of these patients.

The pharynx is the garbage dump of the bronchial tubes and the nasal passages.

- Sir William Osler

REVIEW OF LITERATURE

Chronic pharyngitis is defined as a chronic inflammation of the pharyngeal mucosa in which the pharyngeal mucosa may appear granular or hypertrophied [3, 7]. Although this is a very common clinical situation the underlying etiopathogenesis and case definition especially in terms of duration of symptoms still continue to be controversial[3]. The paucity of literature on this subject compounds to the management of this problem. Chronic pharyngitis is most often defined by its most predominant symptom i.e. persistent sore throat and the presence of prominent lymphoid tissue in pharynx [7].

Chronic pharyngitis is a term frequently used synonymously with chronic tonsillitis, although in reality it is a spectrum of conditions, from chronic inflammation localized primarily to tonsils to generalized inflammation of the whole pharynx [7].

Anatomy of Pharynx

The pharynx is a conical fibro muscular tube that extends from base of skull to oesophagus. It is anatomically divided into:

Nasopharynx – It is the uppermost part of the pharynx that lies behind the nasal cavity from the skull base to the level of the horizontal plane passing through the hard palate.

Oropharynx – Part of the pharynx opposite the oral cavity from the plane of the hard palate above to the hyoid bone below.

Laryngopharynx – It is the lowest part of the pharynx from the level of the hyoid bone to the lower border of cricoid cartilage.

The structure of the pharyngeal wall from within outwards consists of

1. Mucous membrane - The Pharynx is lined by squamous epithelium except the nasopharynx which is lined by columnar ciliated epithelium.

2. Sub mucosa

3. Pharyngobasilar fascia – It is a fibrous sheet, deep to the pharyngeal muscles. It is thick in the upper part where it bridges the gap between upper border of the superior constrictor and the base of skull. Posteriorly, it thickens to form the pharyngeal raphe.

4. Muscular coat – Consists of an outer circular layer made up of three constrictors and a longitudinal layer made up of stylopharyngeus, salpingopharyngeus and palatopharyngeus.

5. Buccopharyngeal fascia - It covers the outer surface of constrictors. Beneath the buccopharyngeal fascia, on the muscular coat is found the pharyngeal plexus of veins and nerves.

Situated in the subepithelial layer are aggregates of lymphoid tissue arranged in the form of a ring which function as barrier to infection in first few years of life. These include the adenoids, paired tubal tonsils around Eustachian tube orifice, palatine tonsils, lingual tonsils, the lateral pharyngeal bands and follicles in the posterior pharyngeal wall. Localised inflammation in any one of these lymphoid tissues is referred by specific terms such as adenoiditis, tonsillitis etc. However when the entire mucous membrane of the pharynx is involved it is termed as pharyngitis. Generally this is accompanied by inflammatory enlargement of the lymph follicles in the tonsils, when it is termed as tonsillopharyngitis [(7)].

Pathophysiology of pharyngitis

Acute pharyngitis is a sudden onset inflammation of the pharynx and is most commonly viral in nature. The exact duration demarcating acute from chronic has not been defined. It is characterized by initial hyperaemia followed by oedema of the mucous membrane and increased secretion from the mucosal glands. Usually it is accompanied by inflammatory enlargement of the lymph follicles in the tonsils, when it is termed as acute tonsillopharyngitis.

Chronic pharyngitis is a chronic inflammation of the pharyngeal mucosa in which the pharyngeal mucosa may appear granular, oedematous or hypertrophied. Here there is proliferation of the connective tissue cells of the pharynx with hypertrophy of the mucosal glands .The hypertrophy of the sub epithelial lymphoid follicles gives it a granular appearance. When the proliferation of connective tissue progresses to a marked degree, fibrous bands and nodules may form. Hyperplasia of the mucous membrane in chronic pharyngitis makes it rigid and thereby causes pain and difficulty in swallowing. The hyperaemia of the small vessels leads to an alteration in the composition and diminution of the mucus secreted. Consequently such patients often complain of dryness of throat and a pricking sensation in the throat which may be associated with a dry harsh cough. There is a tendency of mucus plugs to aggregate on the surface which instead of effortlessly being propelled by the cilia is only expectorated by much coughing or hawking. There is an accumulation of secretions which if allowed to remain, propagates the vicious cycle of irritation and inflammation (8). Occasionally instead of hypertrophy there is atrophy of the mucosa and glands called as atrophic pharyngitis or pharyngitis sicca.

Normal Flora of pharynx

The normal flora of the pharynx consists of a plethora of aerobic and anaerobic organisms. In healthy individuals, the oropharyngeal flora consists of α haemolytic streptococci, non pathogenic *Neisseria* and low numbers of *Streptococcus pyogenes*, *Streptococcus pneumoniae, Haemophilus influenzae, Staphylococcus. aureus*, *Moraxella catarrhalis, Fusobacterium spp.* including *Fusobacterium nucleatum* , members of the genus P*revotella, Porphyromonas,* anaerobic gram positive cocci like *Peptostreptococcus spp*. *Actinomyces israelii, Candida albicans*, adenoviruses and herpes simplex virus [(9)]. In normal individuals, the anaerobes outnumber aerobes 100:1 in the oropharynx [(10)].

Bacteriology of pharyngitis

Viruses are responsible for 85% to 95% of acute pharyngitis in adults. The main aetiological agents are adenovirus and respiratory syncytial virus (RSV).The most important bacterial cause of a throat infection is group A β-haemolytic streptococcus (GABHS), which is responsible for about 10% of sore throats in adults [(11)]. Antimicrobial therapy is of no proven benefit in the treatment of acute pharyngitis due to bacteria other than Group A streptococci, *Corynebacterium diptheriae* and *Neisseria gonorrheae.* Despite this there is an over prescription of antibiotics leading to emergence of antibiotic resistance, besides exposing the patient to potential adverse effects of such therapy [(12)].

The microbiological profile of chronic pharyngitis is not well documented. The bacteriology of chronic pharyngitis on the other hand had been well studied. Group A β - haemolytic streptococci is the most common bacterial pathogen causing chronic tonsillitis. These organisms are believed to reside in the crypts of the tonsils forming micro abscesses which can

cause recurrent episodes of tonsillitis [13]. Other organisms implicated are staphylococcus aureus, streptococci pneumuniae.There are no studies describing the microbiological profile of chronic pharyngitis.

Clinical profile

The clinical profile of acute pharyngitis is fairly similar in children and adults.

The clinical picture in adults is characterized by an abrupt onset of the following:

- Sudden onset of sore throat associated with difficulty in swallowing
- Fever moderate (39-40.5°C)
- Chills maybe present but rigors rare
- Malaise, Headache
- Abdominal pain ,nausea and vomiting may be seen in children

Signs include tonsillopharyngeal erythema, tonsillopharyngeal exudate, softpalate petechiae, swollen uvula and swollen and tender anterior cervical lymph nodes [12].

Chronic pharyngitis on the other hand present with mild persistent throat pain or irritation. They may present with mild difficulty in swallowing with hawking sensation .Clinical examination reveals granular posterior pharyngeal wall .Thickening of lateral pharyngeal wall and edema of the pharyngeal mucosa with stasis of secretions may be present

Etiological classification

Chronic pharyngitis is divided into two types based on the whether a definite etiological agent is detected. The two types are

1. **Chronic specific pharyngitis** - When the condition is secondary to a primary infection by specific micro organisms like *Treponema pallidum*, *Mycobacterium tuberculosis Mycobacterium leprae* and others, this is termed as chronic specific pharyngitis[7]. These micro organisms generally cause systemic disease with involvement of more than one organ system.

2. **Chronic non specific pharyngitis** - When no definite pathogenic organism is identified as etiology of pharyngitis it is termed as non specific pharyngitis. Patient presents with history of long standing throat discomfort without any evidence of specific etiological factor, and often with little to suggest on clinical examination apart from prominent lymphoid tissue in the pharynx. Various possible etiological factors suggested are heavy smoking, occupational irritants, chronic sinusitis with post nasal drip, mouth breathing, acid reflux and poor dental hygiene. Evaluation of such patients requires a careful history and detailed examination of the nose, oral cavity, oropharynx, larynx, pharynx and neck. Exclusion of malignancy is the most important aspect of managing these patients [7].

Chronic specific pharyngitis

Syphilis:

Syphilis is a sexually or congenitally transmitted infection caused by spirochaete, *Treponema pallidum* which progresses through primary, secondary and tertiary stages. It is the secondary stage which usually gives rise to pharyngeal symptoms [14].

The primary stage is characterized by a chancre most commonly seen in the genital area although occasionally it can affect extra genital areas such as the pharynx [7]. The lesion begins as a papule which breaks down to form a painless ulcer with indurated margins. The ulcer persists for two to six weeks and resolves spontaneously [13].

Secondary syphilis occurs four to six weeks after the primary lesion and is characterized by fever, malaise, headache and mucocutaneous rash. Sore throat occurs in about 30% of such cases [6]. The pharynx appears inflamed and hyperaemic with lesions classically described as mucous patches or 'snail-track' ulcers. These ulcers are more common in the oral cavity than in the oropharynx and are usually covered with a grayish white membrane which when scraped has a pink base. In contrast, to other membranes, scraping away of this membrane does not lead to bleeding [5]. This stage lasts for a few weeks and the lesions are highly infectious.

Tertiary syphilis develops in 30 % of untreated patients 5-25 years following primary infection. It typically manifests as a gummatous lesion involving any part of the body. It can occur in the hard palate, nasal septum, tonsil and posterior pharyngeal wall as a granulomatous lesion that begins as a nodule and breaks down to form an ulcer [14].

Serological tests are the mainstay of diagnosing syphilis. The VDRL test (Venereal Disease Research Laboratory test) is the screening test used and the TPPA (Treponema Particle Agglutination Test) which uses treponemal antigens is used as a confirmatory test [13]. Once diagnosed, treatment is with Benzathine penicillin, 2.4 million units, intramuscular given as a single dose for primary or secondary syphilis. In tertiary syphilis, crystalline penicillin is preferred at dosages recommended by the CDC in 2006 [14].

Tuberculosis

The pharynx is a common site for tuberculous infection in children. They usually present with an asymptomatic focus in the tonsil or adenoid with associated cervical lymphadenopathy. Secondary TB affecting the pharynx usually occurs in highly sputum positive patients with

massive cavitating pulmonary lesions. It manifests as multiple, shallow, painful ulcers in the pharynx or oral cavity. The problem has been compounded in recent years with rise of the HIV epidemic and the resurgence of TB closely following the epidemic [7]. Diagnosis is made easily because of clinical and radiological correlation with pulmonary TB. Microscopic examination of the stained smears remains the gold standard of diagnosis despite introduction of newer tests like PCR. The treatment is the anti-tuberculous regime as per the RNTCP (Revised National Tuberculosis Control Programme) category and protocol.

Toxoplasmosis

Toxoplasmosis is a rare disease caused by *Toxoplasma gondii* which in the early stage can manifest as sore throat with malaise, fever and cervical lymphadenopathy. In India, Sundar et al have reported a prevalence of 26% in healthy blood donors [15]. Toxoplasmosis occurs both in the immunocompetent and the immunocompromised and as expected is more severe in the latter. Organs most commonly affected are the CNS, heart and eye especially in children with congenital infection. Pharyngeal infection with this agent is rare and clinically resembles infectious mononucleosis [16]. Kardon et al found that out of 22 tonsillar granulomas subjected to histopathological evaluation only one was due to toxoplasmosis [17].

In immunocompetent individuals the disease is usually self limiting .However in immunocompromised patients it can lead to encephalitis and space occupying lesions in the brain. The former group of patients needs no treatment but the latter may need combination therapy with pyrimethamine and sulphadiazine or azithromycin [7].

Leprosy

Leprosy is a chronic condition caused by *Mycobacterium leprae* which not only affects the skin and peripheral nerves, but also the respiratory mucosa and eyes. Isolated involvement of the pharynx is rare, it usually occurs secondary to nasal infection where it gives rise to granulomatous lesions ulcerating and healing with fibrosis. Treatment is multidrug therapy with dapsone and rifampicin [(7)].

Scleroma

It is a chronic infection caused by *Klebsiella rhinoscleromatosis* which primarily affects the nose and from there can spread to the pharynx where it produces granulomatous lesion which heals by scarring [(7)].

Candidiasis

Candida albicans which is usually normal pharyngeal flora may become pathogenic when there are some local or systemic changes in the host. The most common symptom is pain and dysphagia while examination reveals small curdy white patch which when removed leaves an erythematous lesion. Candidiasis with local predisposing factors responds to local therapy with nystatin drops and lozenges 1,00,000 units six hourly [(7)].

HIV and AIDS

Both the primary HIV infection and the full blown AIDS syndrome can manifest with pharyngeal involvement. Acute seroconversion can present as fever, pharyngitis, malaise, maculopapular rash and cervical lymphadenopathy similar to glandular fever. Opportunistic infections with Candida, TB, syphilis and CMV are seen. Lymphoid hyperplasia of all the tissues of the waldeyer's ring is seen which if present in the nasopharynx requires biopsy to exclude

malignancy. AIDS defining malignancies like Non Hodgkins lymphoma, Kaposi's sarcoma, Squamous cell carcinoma and Hodgkins lymphoma can also be seen. Treatment is anti retro viral therapy and prophylaxis for opportunistic infections [7].

Chronic Nonspecific Pharyngitis

Laryngopharyngeal reflux

Gastro-oesophageal reflux disease (GERD) is defined by oesophageal symptoms or mucosal damage produced by abnormal reflux of gastric contents into the oesophagus [18].

Laryngopharyngeal reflux (LPR) or reflux laryngitis is one of the most common extra-oesophageal manifestations of GERD [18,19].

Population based studies found that the prevalence of symptoms attributed to LPR was in the range of 15% to 20% [20, 21]. De Vault (2000) and Postma et al (2002) have stated that up to 15% of all visits to the otolaryngology clinics are because of manifestations of LPR [22, 23].

Aetiology of GERD and LPR

The aetiology of GERD and LPR is multifactorial, including oesophageal sphincter function, exposure time, and tissue sensitivity. It has previously been shown that laryngeal epithelium is more sensitive to gastric reflux than oesophageal epithelium. The number of episodes of reflux required to cause disease in the larynx are lower, so that even three reflux episodes (pH 4.0) per week are likely to cause LPR. In contrast as many as 50 episodes per week are needed for GERD to manifest [24, 25]. The basis of this increased sensitivity remains poorly understood, but it is thought that the presence of a low pH in the larynx coupled with pepsin causes a change in the reaction of stress proteins in regard to epithelial damage repair, resulting

in damage not seen so readily in the oesophagus. This allows a minimal amount of gastric reflux to cause LPR.

Clinical features and diagnosis of LPR

Common throat symptoms attributed to LPR include hoarseness, vocal fatigue, frequent throat clearing, nocturnal laryngospasm, chronic cough, dysphagia, and globus. Chronic laryngitis and a difficult-to-treat sore throat are associated with acid reflux in as many as 60% of patients [1, 26-31].

However, many of these symptoms are non-specific and may be found in other conditions such as smoking, allergies and postnasal drip. The presence of the aforementioned symptoms in addition to detection of edema and erythema of the posterior glottis by laryngoscopic examination is considered to be suggestive of LPR. However the controversies in the literature regarding the diagnostic criteria of LPR make the task quite complicated [32-34]. Some studies presume that mucus stasis, erythema, edema, hypertrophy, ulceration, and granulation of the posterior larynx (arytenoids, interarytenoid notch) are sufficient for diagnosis of LPR [24, 35,36] while others suggest that similar pathological changes in posterior larynx may be present among persons without LPR [34, 35, 37].

According to Reulbach study (n=100), signs of LPR were determined in 64% of the community- based cohort of healthy adults over 40 years of age. In the cohort of people he examined, only 12% of them had a completely normal laryngeal examination [37]. Vavricka et al (n=132) and Hicks et al (n=105) have found few signs of LPR in 85-87% of the healthy volunteers [34,35]. The predicament in diagnosing GERD-related laryngeal and pharyngeal abnormalities is that the examination is subjective and based on the experience of the physician

identifying these potentially acid-related laryngeal and vocal fold lesions. Intuitively, this approach may result in considerable variations among physician's diagnosis of reflux laryngitis. This may explain the inconsistent treatment results with proton pump inhibitors (PPI) and anti reflux measures, as the diagnostic criteria used in the first place are subjective and may vary with different physicians.

Thus solving the dilemma of an ideal diagnostic modality for LPR appears as one of the "key stones" in solving the problem.

The LPR scoring system is a self administered tool described and validated by Belafsky et al (2002), which entails patients to use a 0- to 5-point scale to grade the following symptoms:

(1) hoarseness or voice change,(2) frequent throat clearing, (3) excess throat mucus or postnasal drip, (4) difficulty swallowing, (5) coughing after eating or lying down, (6) breathing difficulties or choking spells, (7) troublesome or annoying cough, (8) sensation of something sticking or a lump in the throat, and (9) heartburn, chest pain, indigestion, or stomach acid coming up.

The Reflux Symptom Index score greater than 13 is considered abnormal and suggestive of LPR [(38)]. This scoring system has been widely used by various authors and thus a combination of focused history taking and specific findings on flexible nasopharygolaryngoscopy is the most reliable method of LPR diagnosis.

Treatment

The first approach for management of LPR patients is standard dose Proton pump inhibitor (PPI) therapy combined with lifestyle measures. In the case of adequate response after 3 to 6 months, the dose can be titrated to determine the minimal required maintenance dose [(39)]. In those who do not respond favourably to short term standard dose PPI therapy, it is advisable to

stop PPI therapy, and to perform gastro endoscopic examination, preferably four weeks after cessation of therapy. Antireflux surgery may be considered in patients with a previous good symptomatic response to PPI therapy who require chronic PPI therapy, or in patients with an insufficient response to PPI therapy in whom a convincing relationship between reflux and symptoms or lesions has been demonstrated. Despite all these measures many authors have reported treatment failures. This could be because of non adherence to lifestyle modification measures. Recently, Beaver and Karow have suggested that patients with allergy or silent laryngopharyngeal reflux in addition to pre-existing laryngotracheal inflammation may be more susceptible to prolonged airway inflammation when an infection with any of the atypical organisms like Mycoplasma, Bordetella or Chlamydia occurs [(4)].

Postnasal drip secondary to allergic rhinitis and chronic sinusitis

Allergic rhinitis and chronic rhino sinusitis are the other common causes of persistent sore throat. .Allergic rhinitis is an IgE mediated condition in which mast cells and basophils respond to the triggering stimulus by inducing degranulation and release of inflammatory mediators such as histamine. These are responsible for the typical symptoms of nasal congestion, excessive sneezing and watery nasal discharge. Occasionally it may present only as nasal stuffiness and a persistent postnasal drip with constant hawking sensation. Chronic sinusitis especially of the posterior group of sinuses usually presents as postnasal drip, with hawking and even halitosis. Evaluation of any patient with chronic throat irritation entails a thorough history of nasal symptoms and a rigid nasal endoscopy to look for signs of sinusitis. Endoscopic evaluation is the key to diagnosis of chronic rhino sinusitis as it provides precise information on quality of nasal mucosa and presence of secretions and discharge [(40,41)]. X-ray of the paranasal

sinuses gives a high false negative rate [42] and cannot be considered reliable to rule out sinusitis especially in mild cases of sinusitis.

The treatment of allergic rhinitis entails anti allergic measures along with steroid nasal sprays. Chronic sinusitis is of multifactorial aetiology comprising a vicious cycle of pathophysiological and anatomical factors. Treatment is aimed at reducing mucosal inflammation, controlling infection and restoring aeration of nasal and sinus mucosa. If symptoms continue functional endoscopic sinus surgery should be considered [2].

Occupational and environmental irritants

Environmental pollution with plethora of pollutants is a known irritant for the respiratory and pharyngeal mucosa. Workers in certain occupations like printing and dye industry, automobile industry are predisposed to pharyngeal irritation.

Printing workers are exposed to panoply of potentially toxic substances, including pigments, inks, solvents, resins, driers, plasticisers and wetting agents [43] which might have a direct irritant effect and produce chronic pharyngitis or sinusitis. Sama et al (1997) observed that in the automobile industry compounds containing chlorine, chromium, nickel and sulfur are mucosal irritants [44].

Dental sepsis

Chronic periodontitis or inflammation of the periodontal membrane is a condition frequently seen in adults, usually smokers where an abnormal pocket develops between the tooth and gum with deposition of debris and pus. This may manifest as bleeding, halitosis, foul taste

and throat irritation. Treatment is improved oral hygiene with surgical removal of pocket wall and diseased tissue [45].

Smoking

Tobacco consumption and smoking including passive smoking are direct irritants to the oral, pharyngeal and respiratory mucosa. In addition to chemicals such as 4-Aminobiphenyl Benzene, Cadmium, Chromium, 2-Napthyalmine and Nickel which are known carcinogens, it has other constituents like Benzoantracene and Benzopyrene which are mucosal irritants [7].

Despite a detailed evaluation, the most common clinical scenario is an individual with sore throat in whom no etiological factor is discernable .This in turn renders treatment difficult or ineffectual as the primary cause remains undiagnosed [5]. Such people are often thought to be malingering and even referred to the Psychiatry department for assessment [46].

In recent times there have been reports of non-streptococcal agents such as *Chlamydophila pneumoniae*, *Mycoplasma pneumoniae,* anaerobic bacteria such as *Fusobacterium necrophorum*, which are potentially treatable by currently available antibacterials – playing a role in chronic pharyngitis .It has not been established whether they simply act as co pathogens or as primary etiological agents [5,6, 47]

Fusobacterium necrophorum

Fusobacterium necrophorum is an anaerobic non-sporing, gram negative bacilli which belong to the family *Bacteriodiaceae*. Their nomenclature as fusobacteria is derived from their morphology which is spindle shaped or fusiform. Infections caused by these organisms are amenable to treatment with metronidazole [48].

Fusobacterium as normal flora in the oropharynx

Fusobacteria are found in the mouth, genitourinary and gastrointestinal tract of normal individuals [49]. Although various studies have been done to study the anaerobic flora of the mouth, the isolation of Fusobacterium necrophorum has been rare [45]. Moore et al (1982) found no evidence of *F.necrophorum* in samples of gingival fluid whereas Brazier et al (2002) isolated fusobacteria from nine of sixteen healthy adults [50, 51]. Tanaka et al did not isolate *F.necrophorum* from the oropharynx of any of their patients [52]. Most common fusobacterium identified as a normal commensal in all these studies was *F.nucleatum* and *F.necrophorum* was rarely detected. However in a recent study, Jensen et al (2007) detected *F.necrophorum* in the throats of 21% of healthy individuals by PCR techniques [53]. The categorisation of fusobacterium as a commensal or pathogenic organism is difficult to ascertain from literature because of the inconsistent methodologies used for their isolation and identification [54].

Fusobacterium and Lemierre's syndrome

Lemierre's disease is an acute infection where there is thrombophlebitis of internal jugular vein following an acute sore throat. *Fusobacterium necrophorum* and other fusobacteria are important causes of this septicemic illness which presents with metastatic abscesses. This disease was initially noticed in young people by Courmont and Cade in the early part of the last century. Lemierre described in detail the illness as we know it today in 1936 and the syndrome is therefore named after him. Lemierre's syndrome is also known to occur following tonsillitis, pharyngitis, mastoiditis, and infection of the teeth and gums. Lemierre's syndrome was particularly recognized in the preantibiotic era as a complication of pharyngitis with parapharyngeal abscess and was rarely reported in the literature during the 1960s and 1970s,

when penicillin came into common use .This disease had become uncommon in the 'antibiotic era' presumably due to empirical antibiotic therapy, leading to its description as the forgotten disease. In recent decades, however, an increasing number of reports of Lemierre's syndrome in children have been published [55].

Fusobacterium and persistent sore throat

In recent times, Batty et al (2004) have opined that these organisms are responsible for longstanding sore throat in the general population. They isolated *F.necrophorum* from 21% of patients with persistent sore throat. The strains isolated from these cases were very similar in character to the ones that cause Lemierre's disease [6]. Jensen et al found *F.necrophorum* in 49% of patients with chronic tonsillitis as compared to 21% on controls by PCR technique [53]. This recent evidence strongly suggests that *F.necrophorum* has a causative role in non streptococcal tonsillitis. However further studies need to be conducted to substantiate its etiological role.

Chlamydophila pneumoniae

Chlamydia pneumoniae also known as *Chlamydophila pneumoniae* [56] is an obligate intra-cellular bacteria which infects mainly ciliated epithelial cells and alveolar macrophages [57]. The life cycle involves two forms, the elementary body (EB) which is the infective form and the reticulate body which is the form that replicates in the host cell [58].

Epidemiology

Humans are the only known reservoir of Chlamydia infections. Person to person transmission occurs via respiratory secretions [59] especially among close contacts [60] more so in conditions of high relative humidity [58]. *C. pneumoniae* is one of the important causes of infections of the respiratory tract such as sinusitis, bronchitis and pneumonia [61]. It is estimated that roughly about 10% of community-acquired pneumonia cases and 5% of bronchitis and sinusitis cases are caused by this organism. It is said to be involved in the pathogenesis and also exacerbation of asthma and chronic obstructive pulmonary disease [58].

C pneumoniae has also been thought to be the agent responsible for persistent cough both in children and adults [62, 63]. Enough evidence has accumulated to associate this organism with atherosclerosis [64]. *C. pneumoniae* is also implicated in causing late-onset Alzheimer's disease. Asymptomatic infection of the upper respiratory tract persisting for long periods of time has also been documented [58]. It has been reported that *C. pneumoniae* can persist in the nasopharynx for a prolonged period subsequent to acute infection of the respiratory tract. Around 5% of healthy adults and children have been documented to have asymptomatic infection [64].

Re-infection and re-activation are thought to be common. It is estimated that as many as 80% of the world's population would have been infected with this agent at some stage in their life time [61]. Recurrent chlamydial disease may result from either repeated infections or persistence of the organism after unresolved infections. Indeed, the high incidence of chlamydial infections and transient immunity typically observed after infection present difficulties in differentiating between persistent infection and re-infection. Presence of high IgA antibodies to *C. pneumoniae* titres is indicative of persistent antigenic stimulation by an ongoing infection as

IgA antibodies are short-lived [64, 65]. Detection of IgG antibodies cannot be used to indicate persistent infection as these antibodies remain elevated for a long time after recovery from an acute infection.

Diagnosis of Infections

Cell culture is considered as the gold standard for diagnosis of *C. pneumoniae* infections. The microimmunofluorescence (MIF) is the serological reference standard. Enzyme linked Immunosorbent Assay's [ELISA] has been used with variable success to detect IgM, IgG and IgA antibodies to the immunodominant MOMP (major outer membrane protein). Results are similar to those observed by MIF [59, 64].

Role in chronic pharyngitis

The role of *Chlamydia pneumoniae* in chronic pharyngitis is still to be completely elucidated. In a study by Falck et al, 12 patients with longstanding throat symptoms, who were also positive by PCR (polymerase chain reaction) for *C. pneumoniae* were selected for a longitudinal study to determine whether *C. pneumoniae* is an aetiological agent for chronic pharyngitis. Nine of twelve patients showed positive IgA antibodies against *C. pneumoniae*. In eight of the nine patient's positive for IgA antibody, the antibodies persisted over the follow up period of around 2 years [5]. Two patients who were initially negative for *C. pneumoniae* became positive when they suffered a relapse. This showed a positive correlation between persistent symptoms and an increased serological titre towards *C. pneumoniae*. These results support the finding that IgA antibodies are reliable markers for evaluating a reinfection or a persisting infection. The same opinion has been expressed by Blasi et al [65]. The biopsies taken from the

granulations on the posterior pharyngeal wall demonstrated the organism by immunohistochemisty in 9 of their 12 patients. Although their study was limited by small sample size (n=12) and the lack of controls, it showed a causative role of *C.pneumoniae* in chronic pharyngitis [65]. Elevated IgA titres have been considered a reliable marker of chronic infection with *C. pneumoniae* in chronic bronchitis and coronary heart disease [5].

Treatment

Falck et al and others [5] have reported the difficulty in treating persistent *C. pneumoniae* infections with antibiotics. Hammerschlag and others [66] have observed that the finding of *C. pneumoniae* in culture after treatment is a common event in patients who show a clinical improvement. Their patients showed clinical improvement while on antibiotics, and for short periods of weeks to a few months after treatment, but relapsed regardless of the type of antibiotic administered. They found that some patients with persisting infection required multiple courses of antibiotic treatment, and have not found an optimal treatment regimen. Roblin et al. [67] demonstrated that persistence of *C. pneumoniae* is not secondary to the development of resistance to antibiotics. Only actively growing chlamydial forms are affected by antibiotics that penetrate intracellular spaces like the macrolides or tetracyclines. The infectious particle of chlamydia, the elementary body, is metabolically inert and therefore resistant to the action of bacteriostatic antibiotics such as macrolides and tetracyclines. Eradication of *C. pneumoniae* in patients with chronic pharyngitis is seldom achieved by a single course of antibiotic [68] Clarithromycin and erythromycin show good in vitro activity, and so far have been the most commonly employed drugs in the treatment of *C. pneumoniae* infection. Newer macrolides, tetracyclines and fluoroquinolones are other potentially effective drugs although their efficacy has only been shown invitro studies and may not be replicated invivo. Falck et al have suggested

that natural immune mechanisms may in most patients eradicate remaining organisms over a period of time [5].

Mycoplasma pneumoniae

Mycoplasmas are the smallest free living organisms and are included within the class *Mollicutes* ["Mollis=soft, cutis= skin"] [69]. The term '*Mycoplasma'* has been derived from Greek word *mykes* meaning fungus and plasma denoting something that is moulded [70]. Chanock et al proposed the name *Mycoplasma pneumoniae* in 1963 for this organism [71]. They lack a rigid cell wall and hence are pleiomorphic. Though they are free living organisms they require serum for growth. On agar containing media, colonies are produced which are opaque at the centre and translucent in the periphery, producing the characteristic "fried egg colonies" appearance [70].

M pneumoniae causes upper and lower respiratory tract infections in all age groups. Infections are most predominant in the 5-20 year age group. The ciliated epithelial cells and alveolar macrophages are preferentially infected by this organism [72]. Infection occurs by adherence to the host cell through a network of adhesins and other proteins. Production of hydrogen peroxide by this bacterial agent is responsible for the injury or damage to the host cell [73].

Epidemiology

Infection is acquired by inhalation of aerosols. Hence, person-person transmission does occur, especially among the household contacts and in closed communities especially military barracks. M. pneumoniae infects all age groups around the world, but children are more likely to develop pneumonia. Every few years it gives rise to epidemics.

Clinical syndromes caused by *Mycoplasma pneumoniae*

In children above the age of 5 years, upper respiratory tract infections are the most common followed by tracheobronchitis and pneumonia. [73]. Cough is the clinical hallmark of respiratory infections caused by *Mycoplasma pneumoniae* [74]. It is also implicated in exacerbating asthma and chronic bronchitis [70].

Other than infections of the respiratory tract including acute exacerbations of asthma and chronic obstructive pulmonary disease, *Mycoplasma pneumoniae* can cause extra-pulmonary diseases in children also. Most of the extra-pulmonary lesions due to *Mycoplasma pneumoniae* are thought to occur due to immune complex mechanism or due to development of auto-antibodies. These include autoimmune haemolytic anaemia, acute glomerulonephritis, renal failure, tubulointerstitial nephritis, IgA nephropathy and neurological complications such as encephalitis, cerebellar syndrome, polyradiculitis and cranial nerve palsies [73, 74]. Moreover it is thought to cause erythema multiforme, arthralgia and arthritis. It is also suspected to cause digital necrosis in patients with sickle cell disease [73].

Role in chronic pharyngitis

Infection with M*ycoplasma pneumonia* is common in patients who present with hoarseness, throat clearing, globus, and cough, regardless of the duration of the symptoms. In a study by Beaver and Karow et al, 15%of such patients had elevated IgM to M*ycoplasma pneumoniae* [4]. The role of *Mycoplasma pneumoniae* in chronic pharyngitis is yet to be completely elucidated, although it is a known etiological agent of acute pharyngitis [6].

Diagnosis of infection

As mycoplasma lack a cell wall they are not detected by a Gram stain. In most laboratories, the simple but less sensitive cold agglutination test is the mainstay of diagnosis [(75)]. ELISA to detect IgG & IgM antibodies are also used and have demonstrated a sensitivity and specificity of 99%. Culture is cumbersome, slow and has a sensitivity not exceeding 60% in the best laboratories, but it is 100 % specific [(76)]. PCR is more useful in diagnosis especially when cultures are inconclusive. As most laboratories do not have culture facilities and the infrastructure and expertise to perform molecular assays, serological tests are used for diagnosis [(73)].

Chronic pharyngitis is a common clinical condition; however there is a paucity of data on its defining criteria and its etiopathogenesis. Determination of the causative agent and elucidation of its pathogenesis can result in effectual treatment and better patient satisfaction in addition to reducing the economic burden on the health care system.

MATERIALS AND METHODS

Research design

1. This is a descriptive study to determine the clinical and bacteriological profile of chronic pharyngitis.

2. To study the role of atypical organisms in chronic pharyngitis, a prospective case control study design was adopted.

Study duration

The study was performed on patients and controls over a period of 18 months from June 2008 to October 2009 in the Department of ENT and Microbiology. The study was approved by the institutional review board of Christian Medical College, Vellore. All study procedures were performed after obtaining informed consent both from cases and controls as per the form attached in Appendix-A.

Sample size

This study was done on 72 patients The Sample size was calculated using the following formula:

$$\text{Sample size} = 2 * p * q * (z\alpha + z\beta)^2 / d^2$$

This sample size of 72 was arrived at by assuming a difference of 20% in the prevalence of the offending organisms in chronic pharyngitis and normal controls. Batty et al (2004 have demonstrated a prevalence of *Fusobacterium necrophorum* in 21% of cases with chronic sore throat and nil from healthy controls. *C. pneumoniae* prevalence has been noted in 12.9% of

healthy adults by serology [77]**.** In the absence of data regarding the prevalence of these agents in the Indian population with chronic pharyngitis we extrapolated these findings to obtain the sample size of 72. As it was a case control study, an equal number of age and sex matched controls were taken to validate the findings.

Selection of study participants

Healthy individuals aged 17 and above who attended the ENT outpatients with complaints of pain or irritation in the throat for 3 months or more and who were willing were included in the study. A two page comprehensive questionnaire (Appendix-A) was given to each of them at the start of the study. The questionnaire consisted of demographic information and detailed medical history which included asthma, allergies, smoking, alcohol use, occupational irritants and reflux symptoms.

After a comprehensive history each of them underwent a detailed general examination and a thorough otorhinolaryngological examination.

Anterior rhinoscopy was done to look for signs of allergic rhinitis and sinusitis such as pale mucosa, mucoid discharge and post nasal drip, in the absence of which further, rigid nasal endoscopy (RNE) was done to rule out sinusitis.

During oral examination each of their Posterior pharyngeal wall appearance was graded as

1. Congested when it was erythematous in appearance
2. Granular when there was presence of granulations, thickening of lateral pharyngeal bands and edema of the posterior pharyngeal wall is noted.
3. Normal

Indirect laryngoscopy was done to rule out malignancy and also to look for signs of larngopharyngeal reflux. The common findings usually seen in LPR are

1. Congestion of arytenoids,
2. Edema of arytenoids and posterior glottis
3. Interarytenoid granulations and heaping
4. Ulceration in the posterior glottis and arytenoids

Rigid nasal endoscopy was performed by a specialist who was blinded to group allocation. To ensure technical consistency, all examinations followed a standard protocol of subject instructions and tasks and used the same equipment. The Rigid nasal endoscope (Servell 4mm zero degree) was illuminated by a xenon light source. The scopy was done to look for any evidence of sinusitis such as discharge, purulence and any collections.

Patients in whom detailed history taking and thorough clinical and endoscopic examination failed to reveal any cause for pharyngitis were taken as cases for the study.

The inclusion and exclusion criteria for cases were as follows

Inclusion criteria

1. Healthy adults aged 17 years and above with complaints of persistent throat pain or irritation for more than 3 months
2. Normal otorhinolaryngological examination
3. Normal indirect laryngoscopy (IDL)
4. Normal Diagnostic nasal endoscopy (DNE)

Exclusion criteria

Individuals who are/or have

1. Immunocompromised
2. Allergic rhino sinusitis
3. Gastro-oesophageal reflux disease (GERD)
4. Smokers
5. Sinusitis on endoscopy
6. Malignancies.

The study and procedure was first explained to each subject and informed consent was obtained (Appendix-B) by the principal investigator.

Two throat swabs for culture (aerobic and anaerobic) and 5 ml of venous blood for serological testing was collected from all subjects (cases and controls) by the principal investigator for uniformity. The posterior pharyngeal wall was swabbed without touching any adjacent structures. These swabs were immediately transported to the microbiology laboratory and without any delay processed according to the protocol followed for the same. Venous blood was collected in a clotted tube to obtain serum for the serological tests.

Controls were selected patients who presented to the OPD with non throat related conditions such as earlobe repair, migraine or were relatives of patients having no ENT complaints at all. They were age and sex matched with the cases and included in the study if they had no history of throat pain or irritation for 3 months. After informed consent, throat swabs were taken for aerobic and anaerobic culture and venous sample for serological testing. The

samples were promptly delivered to the laboratory where they were plated and cultured as described below.

The laboratory personnel were blinded regarding who were cases and controls.

Culture protocol

Standard aerobic and anaerobic cultures to detect pathogens were performed in the Microbiology laboratory as per the standard current protocol followed for the same. The procedure followed has briefly been described

Sample: Two throat swab samples were taken per case or control

Aerobic culture

For aerobic culture, the throat swab was inoculated onto a blood agar (BA) plate.

Each BA plate was incubated at 37^0 C in an atmosphere of 5% CO_2. The culture plates were read after 24 hours and 48 hours of incubation.

If pathogens were isolated they were reported as soon as they were identified along with the antimicrobial susceptibility pattern.

The pathogens looked for were β-haemolytic streptococci, *Arcanobacterium hemolyticum, Streptococcus pneumoniae, Haemophilus influenzae, Haemophilus parainfluenzae, Staphylococcus aureus*.

Anaerobic culture

For anaerobic culture the swab was inoculated into blood agar (BA), neomycin blood agar (NBA), thioglycollate broth (TG) and Robertson's cooked meat medium (RCM). Following inoculation the media were incubated in an anaerobic atmosphere at 37^0 C for 72 hours. Gram stains were done every 24 hours to detect growth in the thioglycollate broth (TG) and Robertson's cooked meat medium (RCM). The plate media (BA & NBA) were checked after 72 hours. If organisms were observed they were tested for aerotolerance (to prove that they were truly anaerobes). Identification and anti-microbial susceptibility testing was done as per the protocol currently followed.

Serology

Sample: Serum was obtained by collecting 5 ml venous blood from each subject into a clotted tube.

Tests done:

ELISA tests to detect IgA antibodies to *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae* was performed using commercially available kits [Euroimmun AG, Lubeck, Germany].

Procedure in brief:

Diluted patient's serum samples were incubated with antigen coated onto the wells in the ELISA plate. In case the sample was positive, specific IgA antibodies bound to the antigen. For the detection of the bound antibodies, a second incubation was carried out using an enzyme labelled anti-human IgA (enzyme conjugate) catalysing a colour reaction. The intensity of the

colour reaction developed was measured using the ELISA reader. The test was validated and interpreted as described by the manufacturer.

The clinical data and the results of the various microbiological tests were noted in the proforma designed for the same and the results were analysed

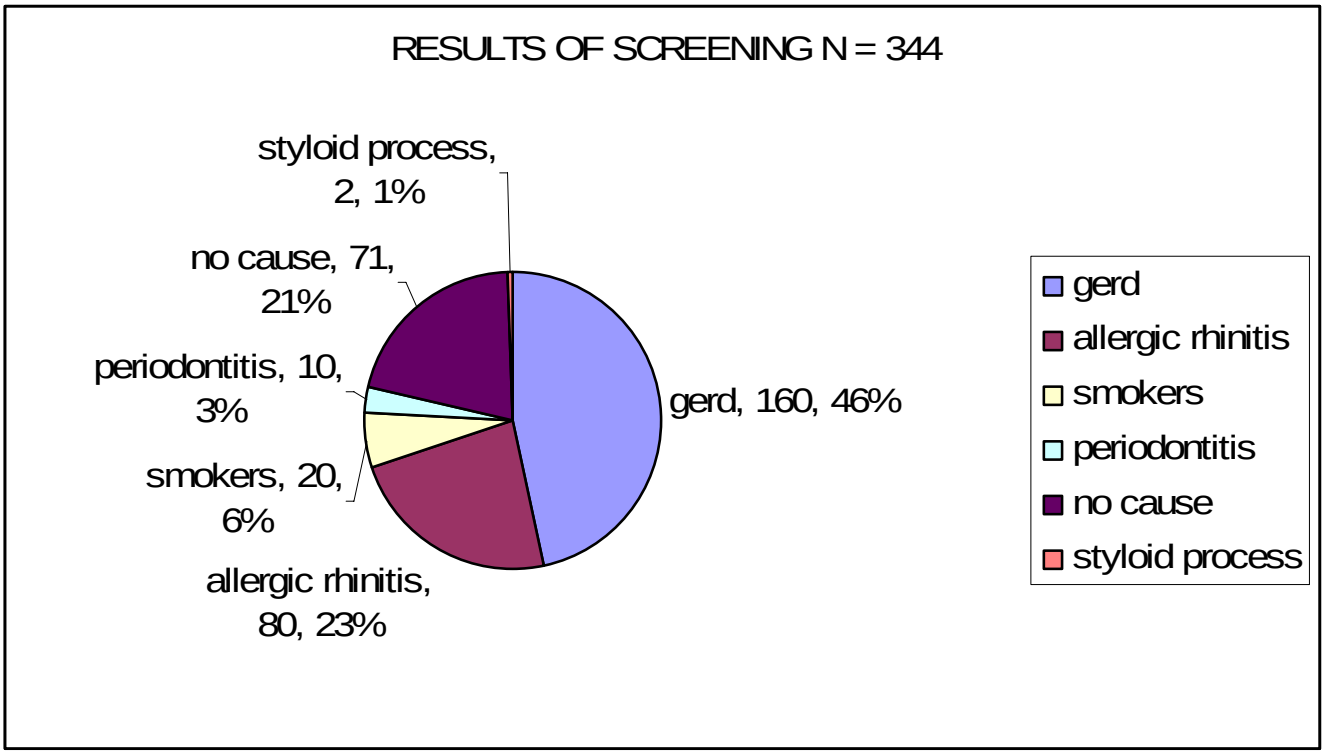
Analysis of data

The data obtained from this study was analysed with SPSS Version 16.0. To calculate statistical significance the chi square test was applied for categorical data and the two sided t test for independent continuous data was used.

RESULTS

This prospective case control study was conducted in the ENT department of a tertiary care hospital. Between June 2008 and October 2009, a total of 344 individuals with clinical features suggestive of chronic pharyngitis were screened. Of these 71 who satisfied the inclusion and exclusion criteria were recruited as subjects. Given below is the clinical profile of the patients screened.

Figure 1: Profile of patients with chronic pharyngtis

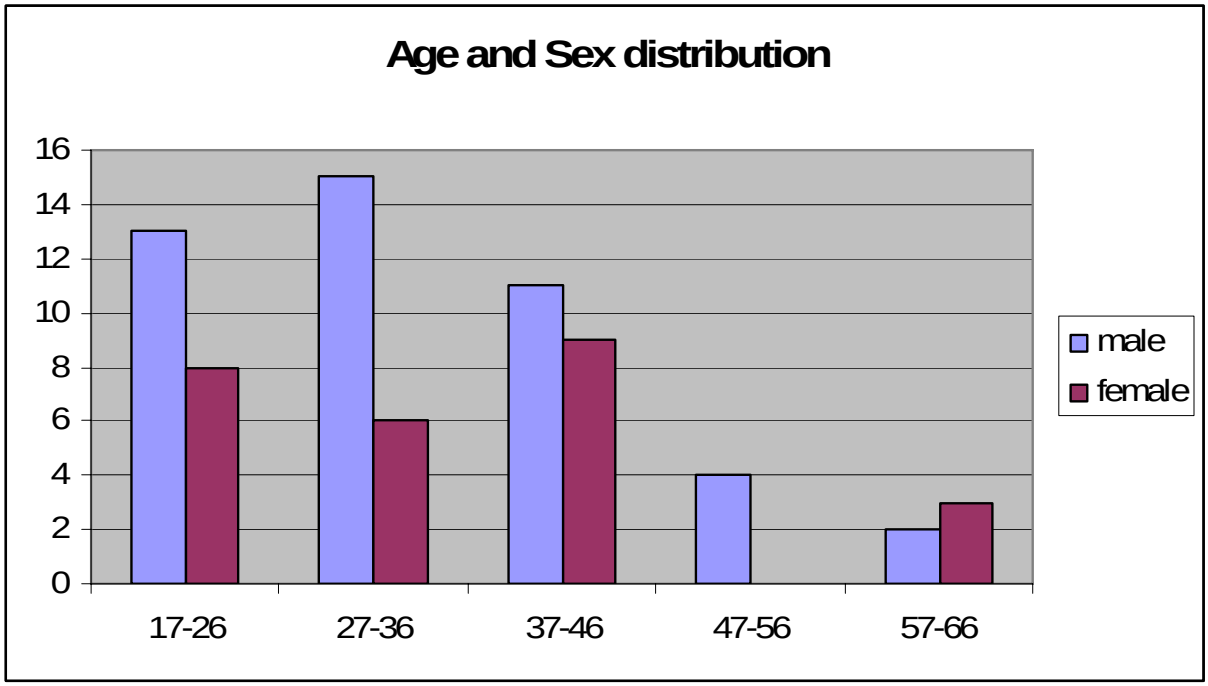


Of the 344 adults with throat pain or irritation who were examined, 160 (46%) had features suggestive of GERD and 80 (23%) of allergic rhinitis. A further 10(3%) had periodontitis, whereas 20 (6%) were smokers and two individuals (1%) had styalgia and hence were excluded. Cases constituted, 71 patients in whom we could not detect any cause for

chronic pharynitis by history, clinical or endoscopic examination, were recruited as cases for the study.

Of the 71 patients recruited, 45 [63.4%] were males and 26 [36.6%] were females. The mean age of the cases was 34.8 years (range 18-65 years). The age wise distribution of the subjects enrolled is as given in the figure below (Fig 1). An equal number of controls who were matched for both age and sex with the patient group were also studied.

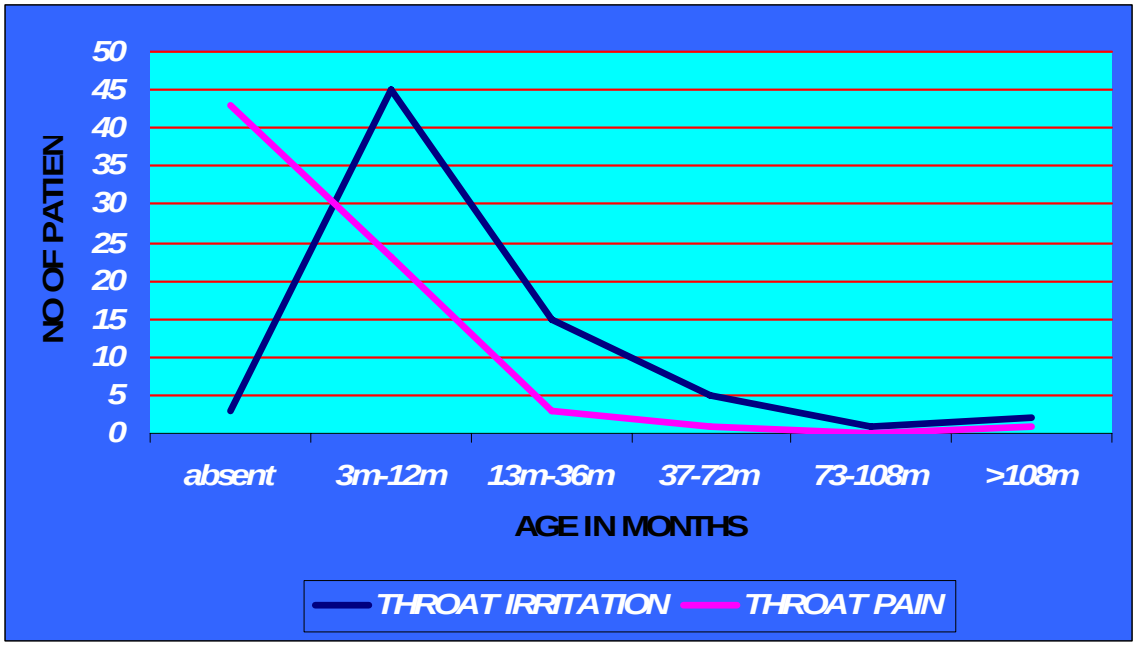
Figure 2: Age and Sex distribution of cases



The age distribution in males ranged from 18-62 yrs with a mean age of 34.13 yrs while inI females it ranged from 18-65 years with a mean of 35.96 years.

Among the males, the most common age group was 27 to 36 while in females it was a decade later in the age group 37-46. Cases recruited were most commonly from the state of West Bengal (43.7) followed by Tamil Nadu (36.6%).and Andhra Pradesh (2.8%)

Figure 3 - Mean duration of symptoms in cases



Mean duration of throat irritation was19.27 months (standard deviation 25.14) whereas mean duration of throat pain was 6.56 months (standard deviation17.8). Chronic throat irritation was the most frequent complaint associated with persistent throat pain in 28 of the patients. Only 3 patients presented with exclusive persistent throat pain

Of the 71 patients almost all of them had taken treatment with medicated gargles. Almost 50 % (35 of 71) of the patients had received more than one course of antibiotics. These antibiotics mainly belonged to the penicillin group.

The results of the various tests performed on both cases and controls are as below

Table 1: Results of tests performed on cases and controls

Test done	Results of tests done			
	Cases (n=71)		Controls (n=71)	
	Positive (%)	**Negative (%)**	**Positive (%)**	**Negative (%)**
Aerobic culture	9	62	9	62
Anaerobic Culture	24	47	36	35
C. pneumoniae IgA ELISA	35	36	16	55
M. pneumoniae IgA ELISA	16	55	16	55

It is clear from the above table that there was no significant difference between cases and controls with regard to growth of aerobes on aerobic culture and serological assays for *Mycoplasma pneumoniae*.The statistical analysis done using the chi square test and the Fisher's exact test further support the lack of significance of aerobic organisms and *Mycoplasma pneumoniae* as probable etiological agents of chronic pharyngitis.

IgA ELISA for *C.pneumoniae* was positive in 35 cases and 16 controls. This difference in IgA ELISA positivity between cases and controls is found to be statistically significant (p=0.001) by the chi square test. Anaerobes were grown in 36 controls whereas only in 24 cases anaerobic organisms could be isolated. Analysis by chi square test has shown this finding to be statistically significant (p= 0.004).

Table 2: List of aerobic organisms isolated from cases and controls

Aerobic organisms isolated	**Cases**	**Controls**
H influenzae	4	1
H parainfluenzae	3	0
S. aureus	2	3
S. pneumoniae	3	2
B haemolytic streptococci	0	3*

* Includes one isolate each of group A, B & C streptococci

Table 2 shows the number and type of aerobic organisms isolated from both cases and controls. 3 patients showed infection with 2 organisms. *H. influenzae* was isolated from four cases and one control. Whereas no β haemolytic streptococci were detected amongst the cases, *H. parainfluezae* could be isolated only from cases.

Table 3: List of anaerobic organisms isolated from cases and controls

Anaerobic organisms isolated	**Cases (28 of 71)**	**Controls (50 of 71)**
Fusobacterium spp.	15	28
Anaerobic GPC (AGPC)*	13	22

Anaerobic GPC: Anaerobic gram positive cocci

Only two types of anaerobes could be identified by culture as seen above (Table 3). Interestingly we observed higher rate of isolation in controls as compared to cases. Among all the *Fusobacterium spp*. isolated from both cases and controls none could be identified as *Fusobacterium necrophorum*. Among the symptomatic individuals 4 who were anaerobic culture positive grew both Fusobacterium and Anaerobic GPC. In contrast 14 individuals among the control group grew both these organisms.

Table 4: Analysis of results of aerobic culture

	Cases	Controls
Aerobic culture positive	9 (12.7%)	9 (12.7%)
Aerobic culture negative	62 (87.3%)	62 (87.3%)
TOTAL	71	71

P value = 1.000

Table 4 In the aerobic culture there is no significant difference (p=1.000) between the cases and controls as shown above.12.7% of both cases and controls showed positive growth on aerobic culture.

Table 5: Analysis of results of anaerobic culture

	Cases	Controls
Anaerobic culture positive	24 (33.8%)	36 (50.7%)
Anaerobic culture negative	47 (66.2%)	35 (49.3%)
Total	71	71

P VALUE = 0.041

A significant difference (P=0.041) in the anaerobic growth culture between the cases and controls is present as depicted in Table 5. In 24 (33.8%) of the cases and 36 (50.7%) controls, anaerobes were isolated from culture.

Table 6: Analysis of *Fusobacterium spp.* growth in cases and controls

Anaerobic organism	Cases	Controls
Fusobacterium spp positive	15 (21.1%)	28 (39.4%)
Fusobacterium spp negative	56 (78.9%)	43 (60.6%)

Table 6 depicts the isolation of *Fusobacterium spp.* in anaerobic culture. A significant difference in the rate of isolation (P=0.018) between the cases and controls is observed. Fusobacteria were isolated in 21 % of the cases and 39% of the controls. It is evident using the Chi-square test that the frequency of isolation was significantly more in controls as compared to cases.

Table 7 Analysis of results of IgA ELISA against *Chlamydia pneumoniae*

	Cases	Controls
Chlamydia positive	35 (49.3%)	16 (22.5%)
Chlamydia negative	36 (50.7%)	55 (77.5%)
Total	71	71

The above table clearly shows the results of the serological tests (IgA ELISA) for *Chlamydia pneumoniae* performed on both cases and controls. IgA antibodies were detected more often among cases than the controls, i.e. 35 (49.3%) of cases were positive in contrast to 16 (22.5%) of the controls. The higher number of positive results among cases in comparison to the same amongst controls was found to be statistically significant (P=0.001).

Table 8: Analysis of results of IgA ELISA against *Mycoplasma pneumoniae*

	Cases	Controls
Mycoplasma antibody positive	16 (22.5%)	16 (22.5%)
Mycoplasma antibody negative	55 (77.5%)	55 (77.5%)
Total	71	71

This table (Table 8) clearly provides information that the IgA ELISA results for individuals with chronic pharyngitis and normals showed no statistically significant difference (P=1.000). An equal number of cases and controls were positive by this test.

Table 9 Correlation between duration of throat irritation and organism isolated in cases

Offending organism	Positive		Negative		Z value	p value
	Mean duration in months	SD	Mean duration in months	SD		
Anaerobic	20.46	27.7	18.6	24.0	-0.11	0.912
Chlamydia	25.94	30.35	12.78	16.79	-2.45	0.014*
Mycoplasma	19.75	29.02	19.13	24.2	-0.76	0.939

The mean duration of throat irritation in months among the cases and its relation to the organism isolated is seen above.

Anaerobic culture positive patients had a mean duration of 20.46 months as compared to anaerobic negative individuals who has a mean duration of 18.6 months. Mycoplasma positive patients showed a mean duration of symptoms of 19.75months whereas it was 24.2 months in mycoplasma negative patients. Both the above values were not statistically significant. In contrast the duration of throat irritation was statistically higher (p=0.014) in cases who were seropositive for Chlamydia (25.9 months) as compared to those were seronegative.

Table 10 Correlation between duration of throat pain and organisms detected in cases

Offending organism	Positive		Negative		Z value	p value
	Mean	SD	Mean	SD		
Anaerobic	11.00	27.03	4.3	10.08	-0.627	0.530
Chlamydia	7.46	22.4	5.6	11.9	-1.18	0.235
Mycoplasma	10.3	29.5	5.47	12.7	-0.237	0.813

Table 10 shows the mean duration of throat pain in months amongst the cases and its relation to a positive result for the three organisms evaluated. No statistically significant (p=0.014) difference was observed in cases who were positive compared to those who were negative for the same.

Table 11- Association between organism isolated and chronic pharyngitis

Offending organism	Odd's ratio	P value
Aerobic culture	1.134	0.821
Anaerobic culture	0.435	0.026
Chlamydia	4.158	0.001
Mycoplasma	0.624	0.304

The odds ratio calculation showed that statistically relevant odds were only observed for positive anaerobic culture and Chlamydia serology. It is to be noted that there is an inverse relation with regard to isolation of an anaerobe by culture in the cases when compared to the controls. In individuals with chronic pharyngitis a direct relation between obtaining a positive serological result and the presence of symptoms is seen.

DISCUSSION

Chronic pharyngitis is a common clinical condition seen by both general practitioners and ENT physicians. Despite detailed evaluation, the most frequently encountered scenario is of a patient with persistent sore throat in whom no obvious cause can be elicited clinically. Although the literature abounds in published reports on the aetiology of acute pharyngitis there is a paucity of data on chronic pharyngitis. This study aimed to determine the clinical and microbiological profile of patients with chronic pharyngitis with special emphasis on the role of atypical organisms such as Mycoplasma pneumoniae, Chlamydia pneumoniae and Fusobacterium necrophorum. These organisms were specially looked for due to recent reports implicating them in chronic pharyngitis [4-6].

In the current study chronic non specific pharyngitis was the most common etiological type encountered among the 344 chronic pharyngitis patient' screened. Laryngopharyngeal reflux was the commonest cause of chronic pharyngitis in this group followed by allergic rhino sinusitis and tobacco consumption. Chronic pharyngitis secondary to tuberculosis, syphilis or any other specific etiological agent was not seen in our study. This was probably because we selected our patients from a general ENT clinic whereas such patients are more likely to report in a general medical clinic with throat symptoms often neglected in the midst of other grave symptoms.

Of the 344 patients screened, in 71 patients no cause was found on clinical and endoscopic examination. These patients were included as cases and an equal number of age and sex matched controls were included in the study.

Having studied the clinical and microbiological profile of patients with chronic pharyngitis in this group, we found this condition to be more predominant in the third decade (mean age male 34.13 years, female 35.96 years) with males more commonly affected than females (male 64%, female 36%). The average duration of throat irritation was 19.27 months and of throat pain was 6.56 months. A granular pharyngeal wall was the most common clinical finding on examination followed by an erythematous posterior pharyngeal wall. In all the above patients the examination of the tonsil and tonsillar pillars was normal.

The aerobic culture from the throat swabs from the posterior pharyngeal wall of both cases and controls revealed a plethora of organisms; however no significant difference was noted between cases and controls. None of the cases grew Group A β haemolytic streptococci in the throat swab culture which is the only known pathogen to have chronic carriage in the pharynx.ref Thus a throat swab for aerobic culture does not seem to be a useful investigation in the evaluation of patients with chronic pharyngitis.

In the evaluation of the anaerobic culture of the throat swabs contrary to the expected results we found that the isolation of anaerobic organisms was significantly more in controls ($p=0.041$) as compared to cases. None of the *Fusobacterium spp.* we isolated could be identified as *Fusobacterium necrophorum*. In recent times, Batty et al (2005) have opined that these organisms are responsible for longstanding sore throat in the general population. They isolated F.necrophorum from 21% of patients with persistent sore throat (6). In the current study we did

not find any such association. Interestingly we found that the isolation of *Fusobacterium spp.* among controls was significantly higher in comparison to the cases (p=0.018). We believe that the increased isolation of anaerobes in healthy controls as compared to subjects with chronic pharyngitis is probably due to the prolonged use of medicated gargles and multiple courses of antibiotics received by these patients prior to treatment at our institute. Anaerobes are commensals in the oral cavity and susceptible to action of oral penicillins. Prolonged use of medicated gargles can cause a change in the oropharyngeal flora.

Serology for *Mycoplasma pneumoniae* did not demonstrate any significant difference. Beaver and Karow et al had demonstrated 15% of patients with chronic pharyngitis to have elevated IgM to mycoplasma pneumoniae. In our study the incidence of mycoplasma in cases and healthy controls was equal. Although a known case of acute pharyngitis its role in chronic pharyngitis needs further evaluation. More sensitive tests like cell culture may help in presenting a clearer picture of the role of this organism. However it is expensive and cumbersome and were beyond the scope of this study.

A statistically significant difference between the cases and the controls was noted only with *Chlamydia pneumoniae* serology .We found the difference in the percentage of subjects with positive serology to Chlamydia to be significantly (p =0.001) higher in patients included as cases (49.3%) as compared to controls (22.5%). An odds ratio of 4.1 (p=0.001) suggested that a patient seropositive for Chlamydia is four times more likely to be a case of chronic pharyngitis than a seronegative patient. In a similar study done by Falck et al they had demonstrated that 75% (9 out of 12) of the patients with chronic pharyngitis had positive IgA antibodies to *C.pneumoniae*. However their study was limited by small sample size (n = 12) and lack of controls [(5)].

Clinically we observed a granular posterior pharyngeal wall in 54 of our 71 patients (73%) as compared to 3 of 71 subjects in our control group (4%). All patients with antibodies against Chlamydia had a granular posterior pharyngeal wall. This granular appearance may be the result of the body's response to infection with *Chlamydia pneumoniae*. Falck et al had demonstrated positive immunohistochemistry for Chlamydia in the granulations from the posterior pharyngeal wall in 9 of the 12 patients in their study [(5)].

We found that the duration of throat irritation was significantly higher (p=0.014) in individuals with chronic pharyngitis who had a seropositive result for c. pneumoniae than in those who were seronegative. In contrast there was no association between the period of throat pain and detection of Chlamydia in cases. No statistically significant correlation between these symptoms and a positive result for mycoplasma serology and anaerobic culture was observed amongst those with chronic pharyngitis.

Asymptomatic carriers of Chlamydia in the respiratory tract are well described .This was first illustrated by Gnarpe et al who found a prevalence of 4.7% in throat of healthy adults using cell culture techniques(68). Similar results were obtained by Emre et al and Block et al who detected *C.pneumoniae* by cell culture in 4.9% and 5% of healthy asymptomatic children (ref). Using serological methods Kern et al have reported prevalence of 12.9% in healthy subjects (ref). In our study 22.5% of the healthy controls had IgA antibodies to *C. pneumoniae* by ELISA. This difference in the prevalence could be because of the use of a different cohort from a different geographical area than employed in their study.

We employed IgA antibody as it is a better marker for the detection of persistent infection. IgG antibodies are long lasting hence an elevated IgG titre does not give any information if the antibodies are due to past infection or due to a persistent one. IgA antibodies being short lived; elevated titres suggest a persisting infection.

There are limited studies on the treatment of these chlamydial infections as the diagnosis is by serology and therefore difficult to assess efficacy of treatment. In the few studies reported [5, 60, 66] treatment has been unsatisfactory in terms of eradication of these bacteria. In the study done by Roblin et al the efficacy of erythromycin and clarithromycin was similar in terms of clinical improvement; however both could not completely eradicate the organism as proved by cell culture methods. In-vitro studies suggest clarithromycin to be better in terms of tissue penetration. This dichotomy in clinical resolution and microbiological persistence suggests that the persistence is not secondary to the development of antibiotic resistance. The persistent nature of chlamydial infections can probably be explained by its unique life cycle. The infectious particle is the elementary body (EB) which after entering the cell develops into the metabolically active reticulate body (RB) [58]. Only the metabolically active chlamydia1 forms are affected by antibiotics that penetrate intracellular spaces like the macrolides or tetracyclines. The infectious particle of Chlamydia, the elementary body, is metabolically inert and therefore resistant to the action of bacteriostatic antibiotics such as macrolides and tetracycline's [68]. This explains the commonly encountered clinical situation wherein patients with chronic pharyngitis show improvement of symptoms for a short period while on antibiotics and for a short period of time thereafter, before they have a relapse of symptoms.

In spite of the various mechanisms described the exact molecular mechanism of chlamydial persistence is not known. There is hope that the mechanism of persistence will soon be unravelled by using tools such as proteomics, and microarrays.

Gnarpe et al and Hammerschlag et al have noted that chronic persistent infection by Chlamydia may be difficult to eradicate with a single course of antibiotics [66, 68]. Falck et al suggest that the immune mechanisms may in most patients eliminate the organism [5]. There are animal studies that propose that induction of mucosal IgA immune response by local immunization may be the future of treatment of chronic pharyngitis (Rodrigues et al 2005).

We suggest that local measures such as medicated gargles for a prolonged duration (3 months) may be a solution to this clinical problem although placebo controlled double blinded trials are needed to further study this.

Beaver and Karow et al [4] have inferenced that individuals with allergies and silent laryngopharyngeal reflux have a more fragile pharyngeal mucosa and consequently more prone to infection with Chlamydia. We in our study have not attempted to study the role of atypical organisms in patients with allergies and Laryngopharyngeal reflux. This could be the subject of another study

Limitations of the study

1. We were unable to identify the species of fusobacteria isolated in our study. Therefore our study does not provide any data regarding whether *Fusobacterium necrophorum* was the predominant species in either cases or controls. Fusobacterium necrophorum was the main offending organism in persistent sore throat in study by Batty et al.

CONCLUSION

Chronic pharyngitis is a common clinical condition seen predominantly in the second and third decades affecting males more commonly than females. Though a paucity of data exists on the case definition of this condition it is most commonly defined by its most predominant symptom i.e persistent sore throat and the presence of prominent lymphoid follicles in the posterior pharyngeal wall. A structured history taking and thorough clinical examination is needed for evaluation of these patients with exclusion of malignancy being the main priority. Pharyngitis secondary to specific disease conditions such as tuberculosis, syphilis, HIV etc are easy to diagnose due to its association with multiple organ systems. Chronic non specific pharyngitis is the most common clinical type encountered in our outpatients. Laryngopharyngeal reflux was the commonest cause of chronic non specific pharyngitis in this study. Such patients have typical history and clinical findings which can be recognised and treatment instituted. Other common causes of chronic pharyngitis such as allergies and asthma, environmental and occupational irritants and dental sepsis should be borne in mind and appropriately addressed.

The common bacteriological agents usually incriminated in causing pharyngitis such as β hemolytic streptococci, staphyococcus aureus and streptococci pneumoniae were not a significant cause of chronic pharyngitis in our study. Infection with chlamydia pneumoniae seems to be a significant etiological factor for chronic pharyngitis. Granulations on the posterior pharyngeal wall should alert the clinician on the possibility of this infection. Granulations on the posterior pharyngeal wall should alert the clinician on the possibility of this infection. These infections are not susceptible to the commonly given penicillin group of antibiotics. Response with macrolide group of antibiotics is good though short lived. This is probably secondary to the

persistence of this organism in a viable but non infectious form on which the antibiotics fail to act. The commonly encountered clinical situation wherein patients with chronic pharyngits show improvement of symptoms for a short period while on antibiotics before they have a relapse of symptoms can be explained because of action of antibiotics on the infectious elementary body and not on the dormant non infectious reticulate body. Studies where long term follow up can be undertaken should be done to assess the best modality of treatment of this infection.

Anaerobic organisms did not seem to be significant etiological agent of chronic pharyngitis in this study .On the other hand a reduction in the commensal anaerobic flora of the pharynx was noted in these patients. This could be due to prolonged use of medicated gargles and repeated courses of antibiotics which the patients had received prior to treatment at our institute. Medicated gargles can also cause a change in the oropharyngeal flora. The inability to speciate Fusobacterium was a limitation of this study and further studies with research laboratory facilities are needed to study the role of anaerobic bacteria in persistent sore throat.

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APPENDIX A- CLINICAL PROFORMA

Date		
Name		
Age		
Sex		
Hospital number		
Place		
Throat irritation	YES/NO	DURATION
Throat pain	YES/NO	DURATION
Dysphagia	YES/NO	DURATION
History of allergic rhinitis		
Bilateral alternating nasal block	YES /NO	DURATION
History of excessive sneezing	YES/NO	DURATION
Post nasal drip	YES/NO	DURATON
History of asthma		
Wheezing	YES/NO	DURATION
Dry cough	YES/NO	DURATION
HISTORY OF ACID REFLUX DISEASE		
Heartburn	YES/NO	DURATION
Regurgitation of sour fluid	YES/NO	DURATION
Frequent throat clearing	YES/NO	DURATION
Hoarseness of voice	YES/NO	DURATION
Globus sensation	YES/NO	DURATION

History of Smoking	YES/NO	DURATION
History of tobacco consumption	YES/NO	DURATION
History of HTN /DM/Asthma	YES/NO	DURATION
	MEDICATION	DURATION
History of previous treatment	YES/NO	
Medical	YES/NO	
Surgical	YES/NO	

EXAMINATION

PULSE

BP

CHEST NORMAL/ WHEEZE/OTHERS

NOSE

PALE NASAL MUCOSA YES/NO

NASAL DISCHARGE YES/NO

ORAL

DENTAL HYGEINE GOOD/FAIR/POOR

ORAL ULCER /LESION YES/NO

TONSIL GRADE 1/2/3

PPW ERYTHEMA/GRANULAR/NOMAL

INDIRECTLARYNGOSCOPY NORMAL/LPR/MASSLESION/OTHERS

RIGID NASAL ENDOSCOPY NORMAL/ALLERGIC RHINITIS /
CHR SINUSITIS/OTHERS

NASOPHARYNGOLARYNGOSCOPY	IF DONE FINDINGS
AEROBIC CULTURE	NORMAL FLORA
	ORG 1 TYPE OF GROWTH
	ORG 2 TYPE OF GROWTH
ANAEROBIC CULTURE	NO ANAEROBES
	ORG 1 TYPE OF GROWTH
	ORG 2 TYPE OF GROWTH
ELISA FOR CHLAMYDIA	VALUE
	NEGATIVE/POSITIVE
ELISA FOR MYCOPLASMA	VALUE
	NEGATIVE/POSITIVE

INFORMATION SHEET

Study Title ; **Evaluation of the bacteriological and clinical profile of chronic pharyngitis with emphasis on studying the potential role of atypical pathogens as etiological agents**

Purpose of research

You have been diagnosed to have long standing sore throat. This study will provide information about possible causes of this condition. We will collect information regarding your complaints. You will undergo an ENT examination during which throat samples and serum will be collected for testing. All these tests are routine and will not result in any extra cost or harm you in any way.

Expected duration of the Subject's participation

If you have long standing sore throat, you will required to come for follow up in the ENT OPD after 15 days.

Description of the procedures

Procedures or therapies for management of disease will remain same (same as any other patient who is not involved in the study, and will include ENT examination, blood investigation and throat sample testing). There are no invasive procedures involved. A set of normal people without any throat complaints will be examined and will have to undergo the same tests and then the results will be compared.

Risks or discomforts to the Subject

As the study doesn't include any trial treatment, there is no extra risk for you due to your participation in the study.

Benefits to the Subject

The benefit of this study to you is that the doctors treating your condition will be better informed regarding the causes of this illness. This may indirectly affect your treatment.

Benefits to others

This will help us to understand the problem better and will help us to find out the most cost effective method of detection of the causes for this condition. We hope that the knowledge obtained will help us to improve the quality of care given to individuals with this type of illness at our centre.

Confidentiality

Your identity will be strictly kept confidential and may be published in a scientific journal. No one other than the treating doctors and the investigators of this study shall have access to your medical records.

Participation

Your participation in the study is voluntary and you are free to withdraw at any time, without giving any reason. Refusal to participate in the research study will not involve any penalty or loss of benefits to which you are otherwise entitled.

Cost to the Subject

There is absolutely no additional cost to you as a result of participation in this study.

APPENDIX B -INFORMED CONSENT FORM

Informed Consent form to participate in a clinical trial

Study Title: Evaluation of the bacteriological and clinical profile of chronic pharyngitis with emphasis on studying the potential role of atypical pathogens as etiological agents

Study Number:

Subject's Initials: _____ Subject's Name: _____

Date of Birth / Age:_____ Phone no.:

Please initial box

(Subject)

(i) I confirm that I have read and understood the information sheet dated _____ for the above study and have had the opportunity to ask questions. [ ]

(ii) I understand that my participation in the study is voluntary and that I am

free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. [ ]

(iii) I understand that the Sponsor of the clinical trial, others working on the Sponsor's behalf, the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the trial. I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published. [ ]

(iv) I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s) [ ]

(v) I agree to take part in the above study. [ ]

Signature (or Thumb impression) of the Subject/Legally Acceptable Representative:_____

Date: _____/_____/_____

Signatory's Name: _____

Signature of the Investigator: _____

Date: _____/_____/_____

Study Investigator's Name: _____

Signature of the Witness: _____

Date:_____/_____/_____

Name of the Witness: _____


BOROSILICATE GLASS
TESTTUBE
BOROSILICATE GLASS

Chronic pharyngitis study - Flow chart for selection of cases and controls